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(56) Documents cited
EP 0450508 A EP 0295092 A EP 0245813 A
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US 4973580 A US 4401662 A

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(54) Wound healing composition

(57) The composition comprises at least 0.1% by weight of one or more glycosaminoglycan oligosaccharides in the size range of from 1 to 60 disaccharide units, particularly hyaluronic acid of molecular weight c.400 – 24,000 Daltons. Preferred compositions comprise homo- and heteromorphic collagen sponges having the glycosaminoglycan oligosaccharides distributed therein.

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WOUND HEALING COMPOSITION

The present invention relates to compositions for use as or in wound dressings or wound implants to assist wound healing.

Numerous compounds and preparations may be applied to or implanted into wounds in order to assist wound healing. They include antiseptics, antifungals, antibiotics and humectants. They may be applied in the form of powders, ointments, films, sponges and the like. In recent years it has been found that certain proteins and polysaccharides can also assist wound healing. Examples of proteins proposed for this purpose are collagen, fibrin, fibronectin and laminin. Examples of suitable polysaccharides are various cellulose derivatives, chitin and the mucopolysaccharides. The mechanism by which the various suggested biopolymers assist wound healing is not clear, but it has been ascribed to a chemical attraction (chemotaxis) whereby the biopolymer increases the local concentration of fibroblasts and endothelial cells at the wound site.

The biopolymers of most relevance to the composition according to the present invention are a class of mucopolysaccharides known as the glycosaminoglycans (GAGs). This class includes hyaluronic acid (HA), chondroitin sulphate, dermatan sulphate, heparin, keratan sulphate and heparan sulphate. Of these, it is hyaluronic acid that has been the most studied from the point of view of its wound healing properties. The background to the present invention will therefore be described with reference to hyaluronic acid only.

Hyaluronic acid is a polysaccharide composed of alternating units of glucuronic acid and N-acetyl glucosamine. The empirically determined formula of each disaccharide unit in naturally occurring HA is roughly $C_{14}H_{20}NNaO_{11}$, giving the HA a molecular weight approximately

equal to 400 times the number of disaccharide units in the HA molecule. The chief characteristic of HA is the high viscosity and elasticity of quite dilute solutions of HA in water. HA occurs in nature associated with collagen in the extracellular matrix of all tissues, where it provides stability, elasticity and water-retaining properties. The viscoelastic properties of synovial fluid and of the aqueous humour of the eye are largely due to the presence of HA in dilute solution. The molecular weight of HA extracted from animal sources varies depending on the source and the method of extraction; reported average molecular weights range from 70,000 to 13 million. The molecular weight distribution for commercially available rooster comb HA is reportedly centred around 400,000, with only minor fractions below 50,000 and above 2 million. Metabolic studies have demonstrated that HA of molecular weight less than about 25,000 undergoes renal excretion, which is consistent with the observed low abundance of this low molecular weight HA fraction in samples obtained from animal sources.

US-A-4141973 (E. A. Balazs) discloses the use in wound dressing compositions of sterile, pyrogen-free, protein free, non-antigenic HA. The disclosure specifically teaches that fractions of HA having an average molecular weight of less than 750,000 are not useful because of their adverse inflammatory activity. The disclosed purification process is used *inter alia* to remove any HA having molecular weight less than 750,000 from the HA obtained by extraction from natural sources. This involves discarding some 80% of e.g. rooster comb derived hyaluronic acid.

EP-A-0138572 (FIDIA S.p.A.) discloses two pharmaceutically active fractions of hyaluronic acid. The first has an average molecular weight between 50,000 and 100,000 and may be used to assist wound healing. The second fraction has a molecular weight between 500,000 and 730,000 and is used for vitreous replacement in intraocular surgery and for intra-articular injections. The lower molecular

weight fraction is obtained by membrane ultrafiltration of a polydisperse aqueous solution of hyaluronic acid. The lower molecular weight fraction is reported to be advantageous for use in wound healing compositions because it forms less viscous aqueous solutions than higher molecular weight HA. It is also reported to have slightly higher cell mobilisation activity than the high molecular weight fraction. However, the specification states categorically that all HA molecules having molecular weight less than 30,000 must be removed from the lower molecular weight fraction by membrane ultrafiltration. The presence of the "inflammatory fraction" having molecular weight less than 30,000 is shown to give rise to problems of wound inflammation.

EP-A-0295092 (Unilever PLC/Unilever NV) discloses cosmetic formulations for topical application to mammalian skin comprising low molecular weight hyaluronic acid oligosaccharides and a cosmetically acceptable vehicle. It is reported that the HA oligosaccharides are small enough to be absorbed readily through the skin and that the HA oligosaccharides have an angiogenic effect, i.e. that they promote the development of blood capillaries. For this reason the compositions disclosed in EP-A-0295092 are said to improve the appearance of aged or wrinkled skin, and especially are said to promote hair growth. There is no suggestion that the HA oligosaccharides can assist wound healing. No medical applications are described for the claimed cosmetic formulations.

It has now been found that GAG oligosaccharides have a surprisingly beneficial effect on wound healing when applied to a wound as or in a wound dressing or wound implant. The term "GAG oligosaccharides" in this context and elsewhere in the specification refers to GAG oligosaccharides in the size range of from 1 to 60 disaccharide units, or mixtures thereof. Since the molecular weight of the GAG oligosaccharide molecules is very roughly 400 times the

number of disaccharide units in the molecule, depending of course on the particular GAG chosen, the above size range of from 1 to 60 disaccharide units corresponds very roughly to a molecular weight range of from 400 to 24,000 Daltons. The beneficial effect observed for the GAG oligosaccharides exceeds that observed for higher molecular weight GAG polysaccharides, and is achieved without adverse inflammatory side-effects. The mechanism of action by which the GAG oligosaccharides assist wound healing is not at present clearly understood.

Accordingly, the present invention provides a composition comprising at least 0.1% by weight of one or more GAG oligosaccharides in the size range of from 1 to 60 disaccharide units for use as or in a wound dressing or a wound implant. Preferably the oligosaccharides are in the size range of from 3 to 25 disaccharide units. The preferred GAG is hyaluronic acid (HA). Preferably the wound dressing composition comprises at least 1% w/w of one or more GAG oligosaccharides, and more preferably at least 5% w/w of the one or more GAG oligosaccharides. The invention encompasses powders, creams, webs, sponges and gels to be applied directly to or implanted into the wound. Other suitable vehicles for the GAG oligosaccharides include synthetic foams or sponges such as those formed from urethane precursors, emollient creams, hydrocolloid dressings, nylon, rayon, cellulose, or oxidised cellulose fabrics and alginate fibres. The invention also encompasses compositions applied to or impregnated on bandages, implants or the like that will be placed in contact with the wound.

The wound dressing or implant composition may preferably also comprise a protein, such as collagen, since as noted above, proteins can assist wound healing. In a particularly preferred embodiment of the present invention the wound dressing or implant composition comprises a protein-based or polysaccharide-based sponge, such as a collagen sponge. One or more GAG oligosaccharides is dispersed in the sponge

continuously and/or in sub-structures within a heteromorphic sponge structure. The latter arrangement permits phasic release of the one or more GAG oligosaccharides as the heteromorphic sponge biodegrades in the wound.

The present invention also encompasses the use of one or more GAG oligosaccharides in the size range of from 1 to 60 disaccharide units for the preparation of compositions as described herein for the treatment of wounds. Preferably, the present invention encompasses the use of one or more GAG oligosaccharides in the size range of from 3 to 25 disaccharide units for the preparation of compositions as described herein for the treatment of wounds.

The GAG oligosaccharides necessary for the practice of the present invention may for example be oligosaccharides of hyaluronic acid, chondroitin sulphate, keratan sulphate, dermatan sulphate, heparin or heparan sulphate. However, the preferred GAG oligosaccharide is hyaluronic acid oligosaccharide.

The GAG oligosaccharides necessary for the practice of the present invention may be obtained by a variety of methods. For example, by molecular filtration or selective precipitation of polydisperse solution of the oligo- and polysaccharides obtained from natural sources by standard extraction techniques. Where the naturally occurring GAG consists mainly of high molecular weight polysaccharides, the fraction of GAG oligosaccharides in the desired molecular weight range may be increased by oxidation with ascorbic acid, treatment with an endoglycosidase, heat shock treatment, ultrasonic disintegration, exposure to ionising radiation, or any combination of these. For example, the hyaluronic acid extracted commercially from rooster combs consists mainly of polysaccharide in the molecular weight range 50,000 to 2 million, corresponding roughly to 130-5000 disaccharide units per molecule. Hyaluronic acid oligosaccharides suitable for the practice of the present

invention may be obtained from this product by incubation with hyaluronidase followed by removal of high molecular weight polymers by precipitation with ethanol or cetyl pyridinium chloride, or by membrane ultrafiltration.

A preferred source of GAG oligosaccharides for the practice of the present invention is the fermentation of microorganisms. Fermentation can provide GAG oligosaccharides more cheaply than extraction from natural sources and with greater molecular weight specificity. For example, fermentation of *streptococcus* bacteria can be used to produce hyaluronic acid in large quantities, the molecular weight of the hyaluronic acid thus produced being on average 50,000 or less (corresponding to 120 disaccharide units or less).

The GAG oligosaccharides obtained by the above methods may be combined with suitable excipients in a bandage, implant, gel or ointment for application to a wound. The composition may contain other active ingredients such as antiseptics, antibiotics, antifungals, humectants, polysaccharides or proteins.

In a preferred embodiment of the present invention the wound dressing or implant composition comprises a protein-based or polysaccharide-based sponge having the GAG oligosaccharides distributed therein. It is envisaged that the protein-based sponge may for example be based on one or more of the proteins collagen, elastin, fibronectin and fibrin. The polysaccharide-based sponge may for example be based on one or more of the polysaccharides hyaluronic acid, alginates, chitosan, chitin, chondroitin sulphate, dermatan sulphate, heparin, heparan sulphate or guar gum. Collagen is a favoured material for forming sponges on account of its low cost, suitable physical properties, insolubility and high degree of bioacceptability. Processes for forming collagen sponges by freeze-drying collagen gels are disclosed for example in US-A-4614794 and in EP-A-0314109.

Processes for preparing collagen sponge having pharmacologically active substances distributed therein are disclosed for example in US-A-4703108.

Where the GAG oligosaccharides are incorporated into a collagen matrix which is subsequently dried as a film or freeze dried to form a sponge a proportion of the GAG is immobilised as a graft copolymer. In order to cross link further the GAG oligosaccharides to the collagen many other methods may be used; for example, exposure of the composite to glutaraldehyde. In this way the proportion of the GAG crosslinked to the collagen matrix may be regulated for optimum wound healing performance. Methods of crosslinking to a collagen matrix are described for example in US-A-4448718.

Protein-based or polysaccharide-based sponges having GAG oligosaccharides distributed therein may be made capable of full degradation and resorption within the body. The sponge matrix is strong and resilient enough to resist collapse; can be cut or will conform to wound shape, will protect the wound bed, will allow the passage of wound fluids, oxygen and other gases and can be replaced by host tissues in such a way that healing is promoted and cosmetic damage minimised. In use, the sponge is applied directly to the debrided wound bed and secured in position by a bandage or the like. Where appropriate, the sponge may be implanted in the wound. Because the sponge is fully resorbable and biocompatible, the problems of irritation and adhesion experienced with more conventional wound dressings are largely eliminated.

The GAG oligosaccharides may be distributed homogeneously in the protein-based or polysaccharide-based sponge. Alternatively, or additionally, the GAG oligosaccharides may be concentrated either in the matrix or in the substructures of a heteromorphic sponge. Heteromorphic protein-based and polysaccharide-based sponges are described and claimed in

our copending patent application filed on the same day as this application under agent's reference G10402P. Briefly, they comprise a porous protein or polysaccharide matrix interspersed with sub-structures. The sub-structures may be formed from material which is the same material as that of the matrix, or they may be formed from another bioabsorbable material. The sub-structures may be milled freeze-dried sponge, powder, films, flakes or other film fragments, aggregates, microspheres, fibres, fibre bundles, or mixtures of these. The advantages of distributing the GAG oligosaccharides in heteromorphic sponges include the ability to control the rate at which the GAG oligosaccharides will be released, including possible phasic release of the GAG oligosaccharides. Moreover, phasic release of more than one kind of GAG oligosaccharides or other pharmacologically active substance may be achieved by incorporation of a mixed population of substructures within the heteromorphic sponge. A further advantage of the heteromorphic sponges is that control over the texture of the sponge permits control of the rate at which cells from the wound invade the sponge.

A preferred material for making the matrix of the heteromorphic sponges according to the present invention is collagen. The collagen may be derived from various natural sources such as animal hide.

Some embodiments of the present invention are described below, by way of example only:

Example 1

Hyaluronic acid oligosaccharides suitable for the practice of the present invention is prepared as follows. Purified, commercially available rooster comb hyaluronic acid is dissolved in hot water at a concentration of 1% w/w. Testicular hyaluronidase is added and the mixture is allowed to react. After sufficient reaction time has elapsed, the

digest is passed through a microfiltration membrane having an average molecular weight pore diameter of 10000. The resulting solution of hyaluronic acid fragments is concentrated by evaporation and then freeze dried.

Example 2

A collagen sponge containing hyaluronic acid oligosaccharides distributed homogeneously therein is prepared as follows. Fibrous collagen is pre-washed to remove the majority of non-collagenous compounds as described in US-A-4320201. The collagen is suspended in deionised water and homogenised in the manner described in US-A-4320201 until the average fibre size is approximately 1 mm. Acetic acid is added to a final pH of 4.0 in order to cause the collagen to swell. Hyaluronic acid oligosaccharides prepared as in Example 1 are added in an amount equal to 1% by weight of the total collagen. After thorough mixing the resulting sponge mixture is degassed, poured into a tray and freeze dried to give a collagen sponge containing approximately 50 mg of hyaluronic acid oligosaccharides per gram of collagen.

Example 3

A collagen film containing hyaluronic acid oligosaccharides distributed homogenously therein is prepared as follows. The sponge mixture of Example 2 is degassed, poured onto flat trays and dried in a stream of warm air.

Example 4

A heteromorphic collagen sponge having hyaluronic acid oligosaccharides distributed in sub-structures therein is prepared as follows. First, the film prepared in Example 3 is crushed into flakes approximately 1 mm across. A sponge mixture is prepared as in Example 1 but without adding any

hyaluronic acid oligosaccharides to the mixture. The film flakes are then added to the mixture, mixed thoroughly, and the mixture is quickly degassed and freeze dried to produce the heteromorphic sponge.

The above examples are intended by way of illustration only. Many other embodiments of the present invention will no doubt be apparent to the skilled reader.

CLAIMS

1. A composition comprising at least 0.1% by weight of one or more GAG oligosaccharides in the size range of from 1 to 60 disaccharide units for use as or in a wound dressing or a wound implant.

2. A composition according to claim 1 wherein the GAG oligosaccharides are in the size range of from 3 to 25 disaccharide units.

3. A composition according to claim 1 or 2 wherein the GAG oligosaccharides comprise hyaluronic acid oligosaccharide.

4. A composition according to any preceding claim comprising at least 1% by weight of the one or more GAG oligosaccharides.

5. A composition according to any preceding claim comprising at least 5% by weight of the one or more GAG oligosaccharides.

6. A composition according to any preceding claim which also comprises a protein.

7. A composition according to any preceding claim which also comprises collagen.

8. A composition according to any preceding claim in the form of a powder, cream, emollient cream, hydrocolloid, gel, web, film or sponge for applying directly to the surface of a wound as or in a wound dressing or implant.

9. A composition according to any preceding claim wherein the composition is distributed in or on a foam, sponge or fabric for use as a wound dressing or implant.

10. A composition according to any preceding claim comprising a protein-based or polysaccharide-based sponge having the one or more GAG oligosaccharides distributed therein.

11. A composition according to claim 10 wherein the protein-based sponge is a collagen-based sponge.

12. A composition according to claim 10 or 11 wherein the one or more GAG oligosaccharides is distributed in substructures within a heteromorphic sponge, thereby providing controlled phasic release of one or more polysaccharides into the wound.

13. A composition according to claim 12 wherein the heteromorphic sponge contains more than one GAG oligosaccharide in a mixed population of substructures.

14. Use of one or more GAG oligosaccharides in the size range of from 1 to 60 disaccharide units for the preparation of a composition according to any preceding claim for use in the treatment of wounds.

15. Use of one or more GAG oligosaccharides in the size range of from 3 to 25 disaccharide units for the preparation of a composition according to any of claims 1 to 11 for use in the treatment of wounds.

Patents Act 1977
Examiner's report to the Comptroller under Section 17 (The Search Report)

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Relevant Technical fields

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(ii) Int Cl (Edition 5) A61F 2/10, A61K 31/73, A61L

Search Examiner

L V THOMAS

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES: WPI, DIALOG/PHARM

Date of Search

22 JULY 1992

Documents considered relevant following a search in respect of claims

1-15

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
X	EP A 0450508 (MEDIOLANUM) - see lines 27-36 page 3 and lines 14-23 page 4	1,2,4-6
X	EP A 0295092 (UNILEVER) - see lines 53-61 page 2	1-5
X	EP A 0245813 (HALFARMACO) - see lines 1-29 Column 1 page 2 and Claims 1 and 9	1,2,4,5
X	EP A 0244298 (SANOFI) - see line 16 page 3 - line 17 page 5, line 26 page 10- line 15 page 11 and line 26 page 14 - line 5 page 16	1,2,4-6, 8,14,15
X	EP A 0214879 (SANOFI) - see line 1 page 4 - line 19 page 5 and Example 5	1,2,4,5
X	US 4973580 (MASULLANI ET AL) - see lines 16-29 Column 3 and lines 4-68 Column 4	1-5,14,15
X	US 4401662 (LORMEAU ET AL) - see lines 16-29 Column 3 and lines 4-68 Column 4	1-5,14,15
X	JP 3015475 (SEKISUI) - see abstract WPI Acc. No. 91-068336	1,3,6,8, 9

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Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

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&: Member of the same patent family, corresponding document.

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